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Regulation of ribulose bisphosphate carboxylase activity *in vivo* by a light-modulated inhibitor of catalysis

(photosynthesis/enzyme regulation/effector regulation)

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ABSTRACT **The activity of ribulose 1,5-bisphosphate carboxylase [RuBPCase; 3-phospho-D-glycerate carboxylyase (dimerizing), EC 4.1.1.39] in leaf extracts of a number of species kept in the dark overnight was found to be very low. This was not the result of a change in the activation state or in the amount of enzyme that could be extracted from "dark" leaves. Rather, in *Phaseolus vulgaris* it was due to an inhibitor of catalysis that occupied the catalytic site of the enzyme. This inhibitor was compartmentalized in the chloroplast and its maximum concentration in both dark leaves and in intact chloroplasts made from such leaves was slightly in excess of the RuBPCase catalytic site concentration. The inhibitor (a phosphate ester) was bound preferentially to the activated form of the enzyme, apparently functioning as a positive effector of activation. Treatment of the enzyme–inhibitor complex *in vitro* with alkaline phosphatase could restore RuBPCase activity. *In vivo*, both the initial rate of disappearance and the final concentration of inhibitor in intact leaves was found to vary with light intensity, and these changes could account for observed light-dependent changes in RuBPCase activity, indicating that light modulation of inhibitor concentration controlled RuBPCase activity. Recovery of activity *in vivo* could be inhibited by 3-(3′,4′,4-dichlorophenyl)-1,1-dimethylurea.**

The *in vivo* activity of ribulose bisphosphate carboxylase [RuBPCase; 3-phospho-D-glycerate carboxylyase (dimerizing), EC 4.1.1.39], the ultimate CO_2-fixing enzyme of all photosynthetic organisms, is known to change in parallel with environmentally induced alterations in photosynthetic capacity, such as with a change in light intensity (1–4) or O_2 concentration (5). In some species, this regulation can be accounted for by the reversible formation of a Mg^{2+}-stabilized carbamate on the large subunit of the enzyme and/or by changes in the pH of the chloroplast stroma (6). However, it has been reported that RuBPCase from several higher plant species was significantly more active *in vitro* after extraction from leaves in the light than from leaves kept in the dark overnight, despite incubation of the low activity form ("dark" form) of the enzyme with optimal concentrations of CO_2 and Mg^{2+} *in vitro* (7–10). With these species, only exposure of dark leaves to light could restore maximum RuBPCase activity. We report here the occurrence of this phenomena in a number of higher plant species, primarily legumes, and we show that the light/dark regulation of RuBPCase activity in *Phaseolus vulgaris* is mediated by light-induced changes in the concentration of an inhibitor binding to the catalytic sites of RuBPCase.

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MATERIALS AND METHODS

Plant Growth. *P. vulgaris* L. (cv. Tender Green) and other species were grown from seed in a controlled environment growth facility under natural illumination and were watered twice daily. Plants also were watered every day with half-strength Hoagland's solution (11).

RuBPCase Measurements. Leaves frozen in liquid nitrogen were extracted in ice-cold 100 mM Bicine, pH 7.8/5 mM $MgCl_2$/5 mM dithiothreitol/0.1 mM EDTA/1.5% polyvinylpolypyrrolidone (≈0.7 cm^2/ml), which had been prepared CO_2-free. An aliquot of this centrifuged extract was immediately assayed for 30 sec at 25°C for RuBPCase activity. This procedure required ≈4 min from extraction to assay, and the measured activity is the "initial" activity, reflecting the *in vivo* activity of RuBPCase. Another aliquot of this same extract was made 10 mM $NaHCO_3$ and 20 mM $MgCl_2$ (10% dilution), incubated for 10 min at 23°C, and reassayed. This activity is the "total" activity, reflecting the maximum activatible activity of RuBPCase. RuBPCase activities were assayed essentially as described by Seemann *et al.* (12). The concentration of RuBPCase in all extracts and in purified preparations was determined by radiolabeling with [2-^{14}C]carboxyarabinitol 1,5-bisphosphate (^{14}CABP) (58 Ci/mol; 1 Ci = 37 GBq) (13, 14).

^{12}CABP–^{14}CABP Exchange. This procedure was carried out essentially as described by Hall *et al.* (15). The ^{12}CABP–^{14}CABP exchange percentage was calculated as the ratio of moles of ^{14}CABP remaining after exchange to the total number of moles of ^{14}CABP bound in the enzyme preparation (×100).

Purification of Inhibitor. Leaf tissue from plants of *P. vulgaris* kept in the dark overnight was frozen and ground to a powder at liquid nitrogen temperature and extracted in 50 mM Bicine, pH 8.0/20 mM $NaHCO_3$/5 mM dithiothreitol/1.0 mM EDTA/5 mM Na ascorbate (1:2, wt/vol). The filtered extract was made to 18% (wt/vol) PEG-4000 and the precipitate was discarded. RuBPCase with bound inhibitor was then precipitated by addition of $MgCl_2$ to 60 mM (16). The pellet was dissolved in buffer and reprecipitated with PEG/Mg^{2+} and finally resuspended in H_2O. This suspension was brought to 3% $HClO_4$, releasing the inhibitor and precipitating most of the protein. The supernatant was then adjusted to pH 7.0 with KOH to remove the perchlorate. The concentrated inhibitor could then be frozen for later use or further purified on a Dowex 1 column by elution with a formic acid gradient (0–8 M).

Phosphatase Treatments. Alkaline phosphatase from bovine intestinal mucosa was prepared in 100 mM Bicine, pH 9.0/1 mM $MgCl_2$/1 mM $ZnSO_4$ at 0.35 mg of protein per ml (188 Sigma units at 25°C). At time zero, this preparation was diluted to a final concentration of ≈0.8 unit/ml in 100 mM

Abbreviations: CABP, 2-carboxyarabinitol 1,5-bisphosphate; RuBP, ribulose 1,5-bisphosphate; RuBPCase, RuBP carboxylase.

Bicine, pH 8.2/20 mM $MgCl_2$/10 mM $NaHCO_3$/5 mM dithiothreitol/0.1 mM EDTA containing RuBPCase that had been exposed to a concentration of either inhibitor or unlabeled CABP equal to or slightly greater than the active-site concentration.

Chloroplast Isolation. Intact chloroplasts were isolated essentially as described by Mills and Joy (17), except the extract buffer was at pH 6.5 and contained 10% PEG-4000 and the underlayered cushion contained 25% Percoll.

Freeze Clamping. Data for Fig. 6 were obtained by using a freeze-clamp apparatus [an improved version of that described by Badger *et al.* (18)] capable of rapidly freeze-killing a known area of a leaf that had been within a temperature controlled photosynthesis cuvette. The cuvette had ambient air flowing through it (340 ppm CO_2, 21% O_2) and the leaf temperature was maintained at 25°C.

RESULTS AND DISCUSSION

Dark/Light Effects on RuBPCase Activity. For *P. vulgaris* and certain other species, the total activity of RuBPCase isolated from leaves of plants grown in the light and kept in the dark overnight was typically only 5–40% of that isolated from similar leaves exposed to full sunlight for at least 1 hr before extraction. This difference in activity was not a consequence of a lesser amount of extractable RuBPCase in dark-treated versus light-treated leaves (data not shown) but rather a result of a decrease in the apparent turnover number (k_{cat}, mol of CO_2·mol of RuBPCase^{-1}·sec^{-1}) of the enzyme (Table 1). With the exception of *Cucumis sativus* (cucumber), all such species were members of the legume family. In contrast, plants such as *Spinacea oleracea* and some other members of the legume family (Table 1) had equally high RuBPCase total activities from either dark- or light-treated leaves.

Dark/Light Effects on RuBPCase Activation State. The complex formed between the reaction intermediate analog CABP and the catalytic site of RuBPCase is much tighter when the catalytic site is in the carbamate form (activated with CO_2 and Mg^{2+}) than when it is not (15). Activated sites form an enzyme–^{14}CABP complex that will not undergo significant exchange with an added excess of free ^{12}CABP in solution, while sites that are not activated come into equilibrium with free CABP within an hour. Changes in the activity of RuBPCase have been correlated with the degree of activation of the enzyme as assessed by this assay (15). We confirmed this result by using purified spinach RuBPCase that had been differentially activated by varying the CO_2 concentration in the suspension buffer (Fig. 1). Assays of activity and activation state of RuBPCase from leaves of *P. vulgaris* or *Vigna radiata* (dark loss of RuBPCase activity) and *S. oleracea* or *Pisum sativum* (no dark loss of activity) are also shown (Fig. 1). When extracted into an activating buffer, the enzyme was apparently fully activated (formed a tight ^{14}CABP complex) regardless of whether it was extracted from a light- or a dark-treated leaf of any of the species. However, the activity was much lower with the "dark" enzyme than with "light" enzyme from leaves of *Phaseolus* and *Vigna*. Apparently, the lower k_{cat} of these species was not the result of inhibition of RuBPCase activation. In fact, the apparent k_{cat} of RuBPCase extracted from darkened leaves of *Phaseolus* declined with increasing concentrations of CO_2 in the suspension buffer (Fig. 2), in contrast to the enzyme from illuminated leaves in which addition of CO_2 (as $NaHCO_3$) increased the maximum activity of the enzyme. These studies show that it is important to distinguish between the activation of the enzyme by CO_2 and Mg^{2+} and the activity as measured in a rate assay.

Basis for Dark Loss of RuBPCase Activity. The activity of dark RuBPCase of soybean can be partially restored by precipitation of the enzyme with $(NH_4)_2SO_4$ (19). Similarly, we found that precipitation of dark *Phaseolus* RuBPCase with PEG-4000 and Mg^{2+} (16) resulted in an increase in the k_{cat} of the enzyme with repeated cycles of precipitation, suggesting the release of an inhibitor from the enzyme. From a total dark k_{cat} of 1.0 sec^{-1}, the activity increased to 7.3 and 13.5 sec^{-1} after the first and second precipitation, respectively. However, this inhibitor appeared to be bound more tightly if CO_2 were present during the precipitation, as the apparent k_{cat} then reached only 3.1 sec^{-1} after the second precipitation. This inhibitor could also be released by treatment of extracts of dark enzyme from *Phaseolus* with mild acid, which removed all RuBPCase activity and released a factor that (after readjusting the pH) inhibited the catalysis of

Table 1. Species examined for dark loss of RuBPCase catalytic activity

Species	% activity
Species with dark loss of RuBPCase activity	
Cucumis sativus	27
Glycine max	36
Phaseolus coccineus	43
Phaseolus lunatus	36
Phaseolus vulgaris	7
Vigna radiata	21
Species with no dark loss of RuBPCase activity	
Camissonia brevipes	125
Geraea canescens	91
Lathyrus odoratus	118
Lens culineris	103
Pisum sativum	96
Spinacea oleracea	120
Vicia faba	106
Xanthium strumarium	139
Zea mays	91

% activity is the ratio (×100) of the total activity (k_{cat}) for RuBPCase extracted from leaves kept in the dark to the total activity of RuBPCase from leaves in high intensity light for 30 min.

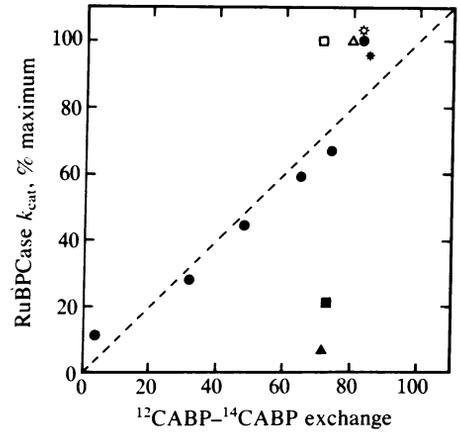

FIG. 1. The relationship between the activity of RuBPCase and the ^{12}CABP–^{14}CABP exchange percentage. Closed circles represent aliquots of purified spinach RuBPCase, which were activated with different concentrations of CO_2. Other symbols represent RuBPCase extracts from leaves either kept in the dark overnight (closed symbols) or in the light (open symbols). In these cases, RuBPCase extracts were incubated under fully activating conditions. Triangles, *P. vulgaris*; squares, *V. radiata*; sunbursts, *P. sativum*. Dashed line is the theoretically expected relationship between activity and the percentage of unexchangeable ^{14}CABP.

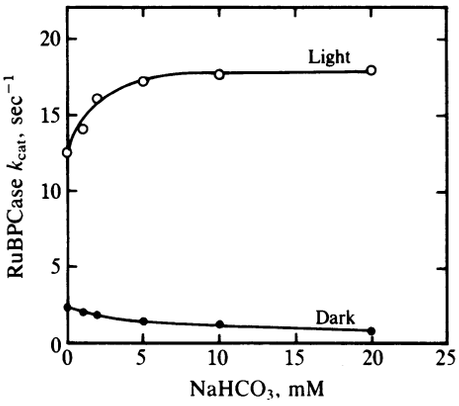

FIG. 2. Effect of CO_2 (provided as $NaHCO_3$) on the k_{cat} of RuBPCase extracted from either dark- or light-exposed leaves of *P. vulgaris*. In both cases, the enzyme was extracted under CO_2-free conditions and $NaHCO_3$ was then added to aliquots of the extracts to the indicated concentration and incubated for 5 min before assay. The activity was determined in assays in which the reaction was started by addition of enzyme and was carried out at saturating substrate concentration. The concentration of RuBPCase was also determined in both extracts.

fresh RuBPCase added to the extract. The inhibitor was acid and heat stable and lost its inhibitory capacity after exposure to alkaline phosphatase, identifying it as a phosphate ester. It was equally effective in inhibiting the activity of RuBPCase from beans or other species, including *S. oleracea, Vicia faba*, and *Rhodospirillum rubrum*.

An experiment demonstrating the presence of the inhibitor in an extract of a dark leaf of *Phaseolus* is illustrated in Fig. 3. The apparent k_{cat} of RuBPCase in an extract of a dark leaf was 2.1 sec^{-1}, ≈10% of that (19.8 sec^1) in an otherwise identical extract of a leaf from the same plant that had been exposed to high intensity light for 1 hr prior to extraction (Fig. 3). A portion of these extracts were brought to pH 2.5 with HCl and heated to 100°C for 1 min. The pH was readjusted to 8.2, and precipitated protein was pelleted by centrifugation. No RuBPCase activity remained in these inhibitor preparations. Another portion of each extract was assayed for RuBPCase protein concentration. Then, increasing volumes of either the dark or light inhibitor preparation were added to a constant volume of the light RuBPCase preparation and it was incubated for 15 min with CO_2 and Mg^{2+}. The final volume of this mixture was held constant by addition of buffer. At a relative inhibitor volume of 1.0 (Fig. 3), the moles of light RuBPCase catalytic sites in the mixture was equal to the moles of catalytic sites present in the added volume of inhibitor extract before acid denaturation. Therefore, the molar ratio of RuBPCase catalytic sites to inhibitor was at this point equal to that in the original leaf from which the inhibitor extract was made. The inhibitor preparation from a dark leaf could reduce the apparent k_{cat} of fresh RuBPCase to about that observed for the RuBPCase present in the original extract (Fig. 3). The much smaller reduction in enzyme activity by the light preparation indicates the essential lack of inhibitor in light leaves of *Phaseolus*, consistent with the much higher catalytic activity of RuBPCase in the original light extract. In addition, we were unable to detect the inhibitor in dark leaves of spinach by this method.

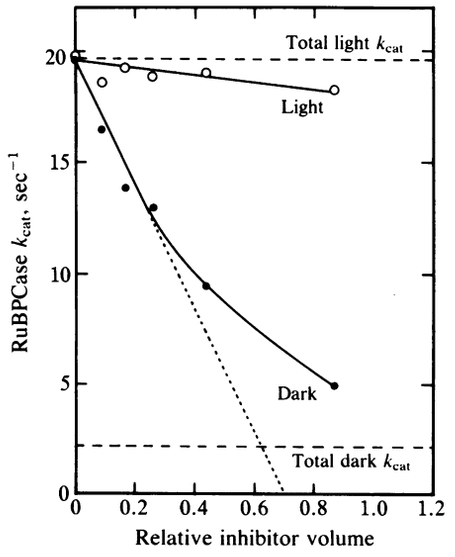

FIG. 3. Effect of (*i*) an inhibitor extract of a leaf of *P. vulgaris* kept in the dark overnight (●) or (*ii*) an inhibitor extract of a leaf of the same plant after exposure to 1000 μmol of quanta·m^{-2}·sec^{-1} for 1 hr (○) on the k_{cat} of RuBPCase. Relative inhibitor volume represents the ratio of the volume of inhibitor extract added to a set volume of an RuBPCase suspension.

Studies with Partially Purified Inhibitor. The observation that much of the inhibitor remained bound to RuBPCase when it was precipitated with PEG/Mg^{2+} in the presence of 10 mM HCO_3^- provided the basis for a technique to separate the inhibitor from many other substances released in the extraction of dark leaves of *Phaseolus* (see *Materials and Methods*) and also indicated that the enzyme–inhibitor complex must be quite stable. Fig. 4 shows plots of enzyme activity assays as a function of catalytic site concentration (assuming 8 catalytic sites per mol of enzyme) conducted at different constant inhibitor concentrations. In the absence of inhibitor, enzyme activity increased linearly with concentration. The slope of this plot is the turnover number per catalytic site. With inhibitor present, the plots were curvilinear at low enzyme concentrations, but when sufficient sites were available to bind all of the inhibitor, the increase in reaction rate with further increases in enzyme concentration was the same as in the control plot (e.g., the plots become parallel). This response is diagnostic of a tight-binding inhibitor (20). Extrapolation of these linear segments back to the horizontal axis indicates inhibitor concentration in the assay (20) (assuming that the inhibitor binds in a 1:1 stoichiometry to the catalytic site of the enzyme). The intercept doubled when the amount of inhibitor was doubled (Fig. 4). The initial

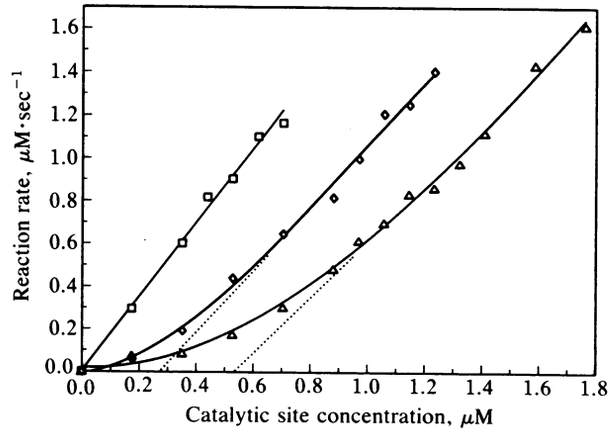

FIG. 4. Plots of the steady-state velocity of RuBPCase as a function of total enzyme catalytic site concentration in the absence (□) or presence of inhibitor (◇, 0.3 μl per assay; △, 0.6 μl per assay). Solid lines are third-order polynomial regressions of the data. Dotted lines are the linear regression of the data above the break.

slope of these curves and their curvature is a function of the dissociation constant of the inhibitor and the fraction of catalytic sites capable of binding inhibitor (20). From these plots, we estimate that the inhibitor dissociation constant (K_d) is $\approx 0.1 \times 10^{-6}$ M.

A plot of activity versus inhibitor concentration with enzyme concentration held constant (Fig. 3) can also be used to estimate the concentration of inhibitor (20). Extrapolation of the tangent to the curve at low inhibitor concentrations to its intersection with the horizontal axis should be the inhibitor concentration that is equivalent to the catalytic site concentration of RuBPCase used in the assay. Results using this method agreed with those above (Fig. 4). Analysis of the data in Fig. 3 indicate that the inhibitor concentration present in the extract of dark leaves was ≈1.4-fold the concentration of RuBPCase catalytic sites in the leaf.

Unequivocal evidence for the interaction of the inhibitor directly with the catalytic site of the enzyme was provided by two observations. First, initial inhibition of RuBPCase activity by inhibitor was competitive with respect to RuBP when the two were added to an enzyme suspension simultaneously (data not shown). The apparent K_i with respect to RuBP was $\approx 0.8 \times 10^{-6}$ M, as compared to 0.4×10^{-6} M for CABP (21). Second, preincubation of the enzyme with inhibitor and $^{14}CO_2$ resulted in formation of a stable ^{14}C-containing complex, which could be separated from free $^{14}CO_2/H^{14}CO_3^-$ by gel filtration (data not shown). This indicates the formation of a tight complex between the activated catalytic site and the inhibitor (22). In control experiments, 87% of the activating CO_2 was trapped by CABP and only 0.4% remained in its absence after passage of the enzyme through a CO_2-free Sephadex G-25 column. The inhibitor–enzyme complex retained 28% of the activator CO_2 (column was preequilibrated with 0.1 μM inhibitor). This complex was thus not as tight as the enzyme–CABP complex. This conclusion was also supported by the observation that inhibitor complexed to enzyme could be displaced by CABP, apparently with retention of the activating CO_2. This provided an explanation for the formation of a tight complex of CABP with dark enzyme (Fig. 1). Preloading of enzyme with CABP prevented inhibitor binding (data not shown).

When inhibitor was added to an enzyme preparation in the absence of RuBP, it took some time to form a tight complex, with an apparent first-order rate constant for this reaction of 0.03 sec^{-1}. Once this complex was formed, the inhibitor could not be displaced by a 10-min incubation with 1 mM RuBP. The inhibition of RuBPCase activity by bound inhibitor was then noncompetitive with respect to RuBP. A similar two-step formation of an enzyme–inhibitor complex has been demonstrated with CABP (21).

Incubation with alkaline phosphatase could restore the activity of RuBPCase complexed with inhibitor (Fig. 5). The rate of this process was much faster than with enzyme bound with CABP. The inhibitor-bound enzyme approached 85–90% of its maximum activity (determined prior to inhibitor binding) after 30 min, whereas similar treatment of the CABP–bound enzyme produced only a very small increase in RuBPCase activity with time. This difference is most likely due to a difference in the dissociation constants of the inhibitor–enzyme and CABP–enzyme complexes, as CABP is rapidly degraded by alkaline phosphatase when not bound to RuBPCase. The degradation of inhibitor bound to RuBPCase was significantly slower when CO_2 was present in the assay buffer than when it was not (data not shown). This is consistent with the results showing that the inhibitor binds more tightly to activated enzyme.

Compartmentation of the Inhibitor *in Vivo*. Experiments with intact chloroplasts were conducted to determine whether this inhibitor was compartmentalized with RuBPcase *in vivo*. The apparent k_{cat} of RuBPCase from chloroplasts isolated from dark leaves (1.8 sec^{-1}) was similar to that from whole leaves, as was the inhibitor to catalytic site ratio (0.9:1). This result can only be explained by postulating that most, if not all, of the inhibitor present in the intact leaves was contained in the chloroplasts. There was essentially no inhibitor present in chloroplasts isolated from illuminated leaves.

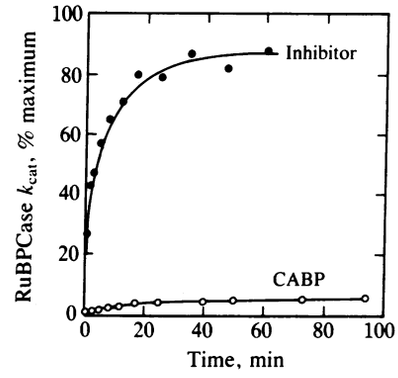

FIG. 5. Time course of the effect of alkaline phosphatase on the activity of RuBPCase that had all catalytic sites blocked with either inhibitor or CABP. The 100% k_{cat} for the enzyme preparations was determined prior to addition of inhibitor or CABP (maximum RuBPCase k_{cat} for inhibitor time course, 20.1 sec^{-1}; maximum RuBPCase k_{cat} for CABP time course, 17.4 sec^{-1}). The enzyme phosphatase preparations were maintained at 25°C.

***In Vivo* Control of RuBPCase Activity.** The activity of RuBPCase of dark leaves of *Phaseolus* could be increased *in vivo* by exposure of leaves to light (Fig. 6*A*). We examined the kinetics and light-intensity dependence of this process and its relationship to inhibitor concentration and RuBPCase acti-

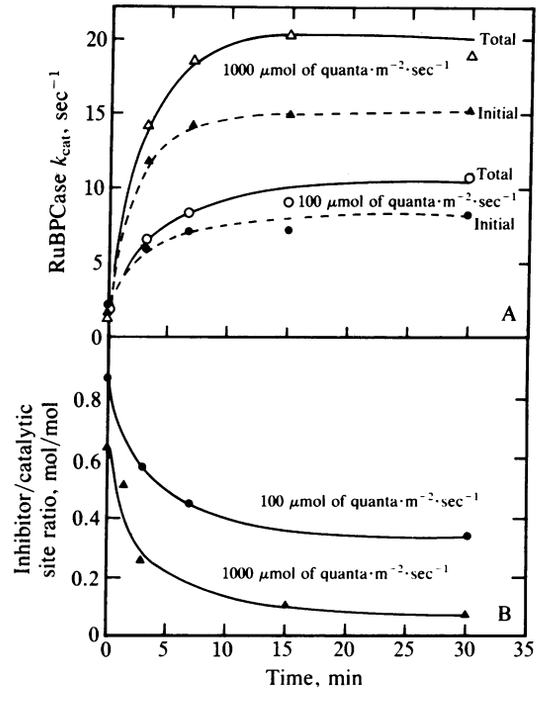

FIG. 6. (*A*) Time course of initial and total RuBPCase activity at two light intensities for leaves of *P. vulgaris* kept in the dark overnight and then exposed to the indicated light intensity at time zero. (*B*) Time course of inhibitor concentration of the same punches as in *A*. Inhibitor/catalytic site ratio (mol/mol) is the molar ratio of the concentration of inhibitor for a particular leaf area to the RuBPCase catalytic site concentration of the same leaf punch and area. Each time point represents a separate leaf.

vation state. The rate of increase of total RuBPCase activity and the final level of activity reached after 30 min in the light were both ≈2-fold higher at 1000 μmol quanta·m^{-2}·sec^{-1} than at 100 μmol of quanta·m^{-2}·sec^{-1} (400–700 nm) (Fig. 6*A*). The total activity reached after 15–20 min at the high light intensity appeared to be the maximal k_{cat} (25°C) for the enzyme. The total activity reached after 30 min at the lower light intensity suggests that about one-half of the catalytic sites were unavailable for catalysis as a consequence of inhibitor bound to these sites. This was confirmed by measurement of the inhibitor/catalytic site ratio in the same leaves (Fig. 6*B*). After 30 min at 1000 μmol of quanta·m^{-2}·sec^{-1}, the inhibitor concentration approached zero, whereas at the lower light intensity the ratio was ≈0.35 mol of inhibitor per mol of RuBPCase catalytic sites.

Despite the large change in enzyme activity, the activation level of RuBPCase (as indicated by the ratio of initial to total activity; Fig. 6*A*) did not appear to change with light intensity or time of illumination. Initial activities were always ≈75% of the total activity. In contrast, other species show considerable light/dark modulation of activation state (1–4). The inhibitor appears to be a positive effector of activation but a negative effector of catalysis.

3-(3′,4′,4-Dichlorophenyl)-1,1-dimethylurea (DCMU) Effect on Inhibitor Metabolism. The basis for the light requirement for modulation of inhibitor concentration was examined using DCMU, a specific inhibitor of photosynthetic electron transport. Discs of dark leaves vacuum-infiltrated with 10 μM DCMU and placed in the light had only slightly higher RuBPCase activity than the dark controls (9.0 vs. 3.5 sec^{-1}), while discs infiltrated with water before exposure to light or DCMU after exposure to light had similar enzyme activities as the light controls (19.0 vs. 21.7 sec^{-1}). These results indicate that steps resulting in the degradation of the inhibitor *in vivo* are dependent on photosystem II electron transport or some product thereof. We hypothesize the existence of a chloroplast enzyme with a specific phosphatase activity that is modulated in some fashion by a product of electron transport.

Concluding Remarks. The evidence presented here indicates that RuBPCase activity in leaves of *P. vulgaris* is at least partially regulated by a chloroplastic reversible tight binding inhibitor of catalysis whose concentration is light modulated. The similarity of response in certain other species (C_3, C_4, CAM) (Table 1 and ref. 9) suggests that this mechanism may be involved in the light regulation of RuBPCase activity in many other species.

The physiological necessity for the control of RuBPCase activity by activator carbamate formation has been questioned, given the effective light regulation of several other photosynthetic carbon reduction cycle enzymes (6). This discovery of another mechanism for the control of RuBPCase activity *in vivo* highlights the need for a better understanding of the regulatory mechanisms involved in the photosynthetic carbon reduction cycle and their role(s) in controlling whole leaf photosynthesis.

In the course of this study, we observed that the inhibitor blocked catalysis by RuBPCase and tended to stabilize the enzyme in the activated state by forming a ternary complex with the activated site. This apparent paradox is not unexpected. Miziorko and Lorimer (6) point out that compounds that bind to and stabilize the activated catalytic site (22–26) must also interfere with catalysis by preventing RuBP from binding to the enzyme, as appears to occur with inhibitor present.

It is interesting that plants possessing this mechanism of regulation apparently maintain their RuBPCase in an activated, albeit inactive, complex in the dark or at limiting light intensities. When the light intensity is increased, the inhibitor is degraded, presumably releasing enzyme that is already in the activated form. Plants such as spinach, which lack this mechanism, also have low RuBPCase activity in the dark but this is because the enzyme is in the deactivated state, and it must then be activated when light intensity is increased. This occurs only slowly in the presence of RuBP (27). The RuBPCase regulatory mechanism that occurs in *P. vulgaris* appears to avoid this problem.

Note Added in Proof. Servaites (28) has recently described a phosphorylated inhibitor of RuBPCase in *Nicotiana tabacum*.

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